

Viral Load Is Reduced and CD4 Count Increased following Multiple Infusions of Cytolin in Subjects with HIV

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ABSTRACT

Background: Cytolin is an anti-LFA-1, mouse, anti-human, monoclonal antibody that binds 4 epitopes of the alpha sub-unit of LFA-1, which is believed to be over-expressed in HIV-infected subjects. Cytolin may reversibly inhibit CD8+ CTL killing of CD4+ T cells; thus, reducing HIV viral load. A phase I study of Cytolin in individuals with HIV showed the drug to be well tolerated. A single 0.1-mg/kg dose was shown to reduce HIV RNA viral load and increase CD4+ and CD8+ T-cell counts at 56 days. This phase Ia/II study was designed to evaluate multiple doses of Cytolin.

Methods: A total of 11 subjects received 4 weekly infusions of 1 of 3 doses of Cytolin (0.3 [n = 4], 1.0 [n = 4], and 2.0 [n = 3] mg/kg); 2 additional subjects also received a single infusion (2.0 mg/kg). Eligible subjects had viral loads > 10,000 copies/mL, CD4+ T-cell counts of 200 to 500 cells/mm³, and a Karnofsky rating of at least 70. Subjects were either treatment naive or were clinically stable on a fixed antiviral regimen for at least 8 weeks and agreed to remain on the fixed regimen during the study period. Safety (incidence and severity of adverse events), HAMA, efficacy (decrease from baseline in viral load, increase from baseline in T-cell enumeration, and effect on triglycerides and cholesterol), and pharmacokinetics (percentage binding, percent-age saturation) were assessed at each visit.

Results: All adverse effects were mild or moderate. A dose-response effect was observed for HIV-1 viral load; larger decreases from baseline in HIV-1 viral load were observed with increasing doses of Cytolin. After 4 infusions, CD4+ T-cell count increased approximately 23% from baseline at day 29 in the multiple-infusion 2.0-mg/kg dose group and correlated with an ~1.0 log decrease from baseline in HIV-1 viral load, which was maintained for at least 50 days from the first infusion, or 28 days from the fourth infusion. Approximately 100% of Cytolin was bound following a single infusion in all dose groups. HAMA reached a maximum accumulation in all dose groups by day 29.

Conclusions: In this study, 2.0 mg/kg Cytolin had the most pronounced effect on increasing T-cell enumeration and decreasing HIV-1 viral load; the effect of multiple infusions lasted at least 50 days and the effect of a single infusion lasted at least 29 days. Additional studies with 2.0 mg/kg Cytolin are underway to determine the most effective dosing regimen.

Methodology:

This study was a Phase Ib/II, single-center, open-label evaluation of three dose levels of Cytolin (0.3 mg/kg, 1.0 mg/kg, 2.0 mg/kg) administered through intravenous (IV) infusion. Eligible subjects had a CD4 T-cell count between 200 and 500 cells/mm³. Subjects were sequentially enrolled and assigned to the lowest of three dose groups in which available slots remained, until 4 subjects were enrolled at each dose level. The study protocol was amended to change the sample size in the third cohort (2.0 mg/kg) from four to five subjects. Three subjects received multiple infusions as before and two subjects received a single infusion of 2.0 mg/kg Cytolin. Each subject was followed for 8 weeks.

The multiple-dose study was designed in two parts to evaluate single-dose safety and pharmacokinetics, and to determine the maximum tolerated dose (MTD) of Cytolin administered by intravenous infusion before proceeding to subsequent multiple-dose treatment. Dose groups administered multiple-doses received a maximum of three repeat doses (i.e., four total doses) administered once weekly by intravenous infusion.

Enrollment at the next higher dose level was only allowed if there were no grade 3 or 4 adverse events in the previous dose level. If there were any grade 3 or 4 adverse events at any dose level, three new subjects were to be enrolled at the same dose level such that nine subjects were to be treated at that dose level. If no further adverse events occurred within one week after treatment, then dose escalation could have proceeded.

Just before and at specified times after administration of each dose of Cytolin, blood was obtained for determination of pharmacokinetic parameters, HIV-1 viral load by ribonucleic acid polymerase chain reaction (RNA PCR), human mouse antibody (HAMA), T-cell enumeration by flow cytometry, and routine laboratory evaluation (hematology, clinical chemistry, and urinalysis).

Diagnosis and Main Criteria for Eligibility:

Subjects were eligible for the study if they met the following criteria:

1. Able to provide written informed consent;
2. Positive HIV-1 enzyme linked immunosorbent assay (ELISA) test;
3. Male or female 18 years of age;
4. Naïve to antiviral drug therapy or stable on a fixed antiviral regimen for at least 8-weeks before study participation, and agreed to remain on the existing treatment throughout the study unless change was recommended by the investigator;
5. Karnofsky performance score of 70;
6. HIV RNA level > 10,000 copies/mL by PCR;
7. CD4+ T-cell count between 200 and 500 cells/mm³;
8. Adequate bone marrow, renal, and hepatic function.

Subjects were not eligible for the study if they met any of the following criteria:

1. DTH skin testing or immunomodulator therapy with 3 months of study entry;
2. Prior Cytolin therapy;
3. History of atopy;
4. Experimental therapies within 60 days of study entry;
5. Prior mouse antibody therapy or positive HAMA at screening.

Subject Demographics

	0.3 mg/kg Multi-dose N = 4	1.0 mg/kg Multi-dose N = 4	2.0 mg/kg Multi-dose N = 3	2.0 mg/kg Single-dose N = 2
Sex				
Male:Female	3:1	4:0	4:0	4:0
Age (Years) mean (SD)	46.5 (8.45)	51.9 (9.26)	46.9 (6.07)	44.2 (8.42)
Years Since Diagnosis mean (SD)	12.5 (5.51)	13.5 (3.70)	8.0 (8.19)	9.0 (11.31)
Weight (kg) mean (SD)	77.8 (5.50)	82.8 (6.70)	69.4 (7.47)	93.25 (15.91)
Cytolin Administration (mg) mean (SD)	23.3 (1.9)	83.7 (6.2)	139.3 (14.5)	186.5 (31.8)

Safety Results:

A total of 125 treatment-emergent adverse events were reported by 13 subjects in all dose-groups. A dose relationship was observed in the multi-dose treatment groups, where the largest number of treatment-emergent adverse events was reported in the 2.0 mg/kg multi-dose treatment group (70, [56%]). The adverse events with the highest incidence (> 3% in all treatment groups were nausea, pain, fever, sinus/nasal congestion, diarrhea, vomiting, headache, facial flushing, myalgia, and rigors. No withdrawals occurred because of adverse events, there were no serious adverse events, and no deaths occurred during the study.

Summary of Safety

	0.3 mg/kg Multi-dose n (%)	1.0 mg/kg Multi-dose n (%)	2.0 mg/kg Multi-dose n (%)	2.0 mg/kg Single-dose n (%)
Evaluable for Safety	4	4	3	2
Treatment-emergent adverse events	21/125 (17%)	29/125 (23%)	70/125 (56%)	5/125 (4%)
Mean number [range] of treatment-emergent adverse events per subject	5.3 [2-10]	7.3 [3-12]	23.3 [14-38]	2.5 [2-3]
Treatment-emergent adverse event intensity				
Mild	7 (33%)	21 (72%)	47 (67%)	0
Moderate	12 (57%)	8 (28%)	23 (33%)	5 (100%)
Severe	2 (10%)	0	0	0
Disabling	0	0	0	0
Serious adverse events	0	0	0	0

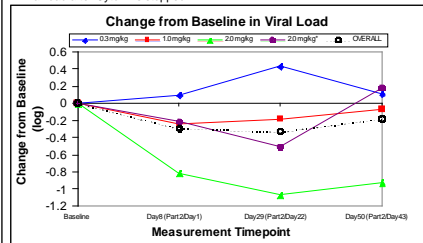
Treatment-emergent adverse event causality	0.3 mg/kg	1.0 mg/kg	2.0 mg/kg	2.0 mg/kg
Pre-existing disease	0	1 (3%)	3 (4%)	1 (20%)
Not Related	12 (57%)	8 (28%)	3 (4%)	0
Unlikely	2 (20%)	2 (7%)	9 (13%)	0
Related	7 (33%)	18 (62%)	55 (79%)	4 (80%)
Possible	7 (33%)	14 (48%)	7 (10%)	0
Probable	0	4 (14%)	48 (69%)	3 (60%)
Definite	0	0	0	0
Discontinuations due to adverse events	0	0	0	0
Deaths on study	0	0	0	0

Summary of Concomitant Antiretroviral Use

Number of subjects not taking antiretroviral medications	3 (75%)	1 (25%)	1 (33%)	2 (100%)
Number of subjects taking antiretroviral medications	1 (25%)	3 (75%)	2 (67%)	0
Subjects taking > 3	0	0	0	0
Subjects taking 3	1 (25%)	3 (75%)	0	0
Subjects taking 2	0	0	1 (33%)	0
Subjects taking 1	0	0	1 (33%)	0

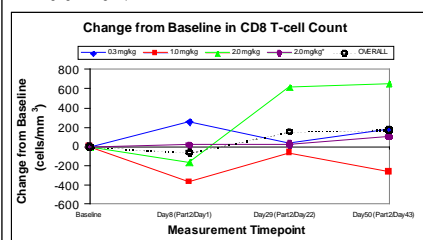
HIV-1 viral load

- A dose response effect observed with the biggest effect in the multi-infusion 2.0 mg/kg dose group.
- The percent decrease in HIV RNA viral load between days 8 and 29 for both the single-dose and multiple-dose 2.0 mg/kg groups was similar (0.29 and 0.25 for the single- and multiple-dose groups, respectively), which suggests that the additional doses of Cytolin may not increase the rate of reduction in HIV RNA viral load.
- The data suggest that additional doses of Cytolin maintain the decrease in viral load after Cytolin is stopped.



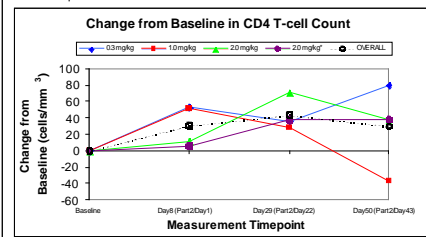
CD8+ T-cell Count

- No clear dose response effect was observed in CD8+ T-cell count.
- The largest sustained increase from baseline was observed in the 0.3 and 2.0 mg/kg dose groups.



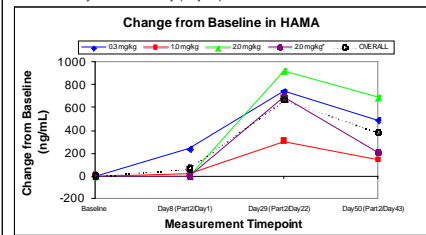
CD4 T-cell count

- CD4+ T-cell count was increased from baseline in all dose groups to Day 29, but no dose response effect was observed.

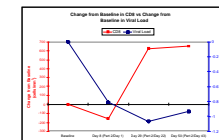
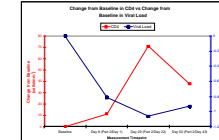


HAMA

- No clear dose-response effect was observed.
- In all dose groups, HAMA increased markedly by Day 29, followed by slight decreases by the end of study (Day 50).



- Pharmacokinetics: Percent Binding (data not shown)**
- Percent binding was approximately 100% following a single infusion in all dose groups.
 - Percent binding was highest in the 1.0 mg/kg dose group and lowest in the 2.0 mg/kg dose group with repeated infusions; this correlated with HAMA, where HAMA production at Day 29 was lowest in the 1.0 mg/kg group and highest in the 2.0 mg/kg group. Thus, the effect of repeated infusions on percent binding may be due to an accumulation of HAMA, which neutralizes Cytolin.
 - Alternatively, the effect of repeated infusions on percent binding may be due to the increased efficiency of the immune system to clear Cytolin with repeated exposure.



HIV-1 viral load correlation to T-cell enumeration

- Further analysis of the 2.0 mg/kg multiple-dose group demonstrates a correlation between change from baseline in HIV-1 viral load and change from baseline in CD4 T-cell and CD8 T-cell counts.
- HIV-1 viral load decreased from baseline as CD4-T-cell count increased from baseline; the maximum increase from baseline in CD4 T-cell count and the maximum decrease from baseline in HIV-1 viral load both occurred on Day 29.
- Similarly, as CD8 T-cell count increased from baseline by Day 29, the maximum decrease in HIV-1 viral load occurred on Day 29.

SUMMARY AND CONCLUSIONS

- Cytolin was safe and well tolerated in this study.
- The data suggest that 2.0 mg/kg Cytolin has the most pronounced effect on increasing T-cell enumeration and decreasing HIV-1 viral load;
- The effect of multiple infusions lasts at least 50 days and the effect of a single infusion lasts at least 29 days;
- The decrease from baseline in HIV-1 viral load observed after multiple 2.0 mg/kg infusions appeared to correlate with the increase from baseline in CD4 and CD8 T-cell counts.